

Isolation and Molecular Identification of New Emergent *Candida Lusitaniae* Isolated from Sudanese Immunocompromised Patients Infected with Oropharyngeal Candidiasis

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Abstract Seventy seven oral swab samples (n=77) were collected in period between august 2007 to may 2008 from hospitalized immunocompromised and HIV patients suspected for Oropharyngeal Candidiasis and admitted in different hospitals in Ed-wiuem state and Khartoum state, Sudan. All samples were inoculated on Sabouraud dextrose agar and identified by colonial morphology, Germ tube test and Vitek2 compact system for biochemical identification and antifungal susceptibility test. Out of 77 oral swab samples collected from immunocompromised and HIV patients, 41 (53.3%) samples showed positive growth of Candida, and 36 (46.7%) samples showed negative growth. The identification showed that out of forty one positive cultures, 32 isolates found as *Candida albicans* (78%), while nine samples (n=9) appeared as non-*Candida albicans* (22%) and found as *Candida lusitaniae* according to GTT and Vitek2 Compact identification. Then DNA was extracted from all non-*Candida albicans* isolates and DNA sequencing was carried and D1/D2 region were determined using NL1 primer. DNA based identification showed that all nine (n=9) GTT negative isolates were *Candida lusitaniae* (Anamorh *Clavispora lusitaniae*). This study documented that there are new emergent species of Candida should be considered when dealing with specimen collected from patients suspected for yeast infections. Our results provide useful information that *C. lusitaniae* can be isolated as well as other Candida species from immunocompromised patients in Sudan.

Keywords: immunocompromised, HIV, *C. lusitaniae*, DNA sequencing, Sudan

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1. Introduction

Candida lusitaniae, is emerging as an opportunistic pathogen in immunocompromised patients. This yeast is generally resistant to amphotericin B and may show therapeutic difficulties. *C. lusitaniae* may be misidentified when detected in blood or other body sites as many other fungal species, including *Candida parapsilosis*, *Candida tropicalis*, and even *Saccharomyces cerevisiae*. *Candida lusitaniae* is considered an opportunistic pathogen, causing several infections primarily in immunocompromised patients [1-6]. The rare non-albicans *Candida* species such as *Candida lusitaniae* has emerged during the last 20 years as an important nosocomial pathogen. [7]. From the first documented case of infection in 1979, amphotericin B resistance has been frequently reported. [7]. The abnormal antifungal susceptibility profiles of some of non-albicans *Candida* species involved in human pathology, their rising

contribution to invasive infections, make their identification to the species level essential for epidemiological investigations and for optimizing therapy and patient management [8]. Although several factors may cause the development of oral candidiasis in immunocompromised patients and different *Candida* species were isolated, but the differential identification of new *Candida* species not yet performed. Therefore the objective of this study was to isolate and identify new species of *Candida* in oral cavity of immunocompromised patients in Sudan.

2. Materials and Methods

2.1. Collection of Specimen and Isolation of Yeasts

Oral swab samples (n=77) were collected in period between august 2007 to may 2008 from hospitalized immunocompromised and HIV patients suspected for

Oropharyngeal Candidiasis and admitted in different hospitals in Edwuiem state and Khartoum state, Sudan.

All specimens were inoculated into Sabouraud's dextrose agar incubated at 37°C for 24 hour. After incubation time all colonies resembling the growth of *Candida* were examined depending on colonial morphology and Gram stain result.

2.2. Germ Tube Test (GTT)

3 ml of human serum was pipette in small test tube, the serum was inoculated with a yeast colony from the culture by using sterile wire loop ,the tube was incubated at 37 C for 2 hrs, then preparation was examined under microscope.

2.3. Identification and Antifungal Susceptibility Test by Vitek 2 Compact System

For Vitek 2 identification, stored yeast were sub-cultured on Sabouraud dextrose agar plates and incubated at 37°C for 24-48 hr. For the preparation of the yeast inoculum, yeast cells were suspended into 3 ml of a sterile saline solution (0.85%) to achieve a turbidity equivalent to 1.80 to 2.20 McFarland turbidity standard, 280 µl of inoculum suspension was transferred to test Card (YST for identification and AST for antifungal susceptibility) and the cards were processed on the Vitek 2 Compact system, according to the instructions of the manufacturer (bioMérieux, Hazelwood, France).

2.4. DNA Extraction

Small portion of colonies was placed in 1.5-ml eppind of tube with 500 µl of lysing solution (100 mM Tris-HCL [ph 8.0], 30 mM EDTA [ph 8.0], and 0.5% sodium dodecyle sulfate) and incubated for 15 min at 100°C the supernatant was transferred to a new tube and the supernatant was extracted with phenol- chloroform- isoamyl alcohol). Then, the samples were extracted with chloroform-isoamyl alcohol) and the DNA was precipitated with 100% and 70% ethanol. The DNA pellet was dried and resuspended in 30 µl of TE buffer and stored at -20c until use.

2.5. DNA Sequencing

The large subunit of spacer sequence Domain 1 and Domain 2 (D1/ D2 LSU) including the 5.8 S rDNA was sequenced commercially using primers NL1 and NL4. The sequences of the two primers were 5'-GCATATCAATAAGCGGAGGAAAAG -3' and 5'-GGTCCGTGTTTCAAGACGG -3', respectively.

3. Result

3.1. Mycological and Biochemical Identification of Yeast Isolates

Out of 77 oral swab samples collected from immunocompromised and HIV patients, 41 (53.3%) samples showed positive growth of *Candida*, and 36 of 77 (46.7%) samples showed negative growth. The

mycological identification shoed that out of 41 positive yeast growths, 32 isolates were found as *Candida albicans* (78%), while nine cultures appeared as non-*Candida albicans* (22%) according to GTT and Vitek2 Compact identification (Figure 1). The nine isolates of non-*Candida albicans* were identified as *C. lusitaniae* by Vitek2 identification system with different identification percentages (probability %) and excellent identification levels (Table 1).

3.2. Antifungal Susceptibility Results

Candida lusitaniae isolates were subcultured and examined for antifungal susceptibility using AST cards of Vitek2 Compact system against four common antifungals (Flucytosine, Fluconazole, Voriconazole and Amphotericin B). All species were resistant to Amphotericin B with MIC ≥ 2 and sensitive to Flucytosine, Fluconazole, Voriconazole with MIC ≤ 1 , ≤ 1 and ≤ 0.12 respectively (Table 2).

3.3. Molecular Identification Results

DNA based identification showed that all nine (n=9) GTT negative isolates were *Candida lusitaniae* (*Anamorh Clavispora lusitaniae*). The Phylogenetic tree of *Candida lusitaniae* isolates obtained on this study, based on 26SrDNA sequences of D1/D2 LSU region, was constructed using data from Oropharyngeal Candidiasis patients samples. Phylogenetic tree showing the relationships among *Candida lusitaniae* isolates and other *Candida* species (Figure 2).

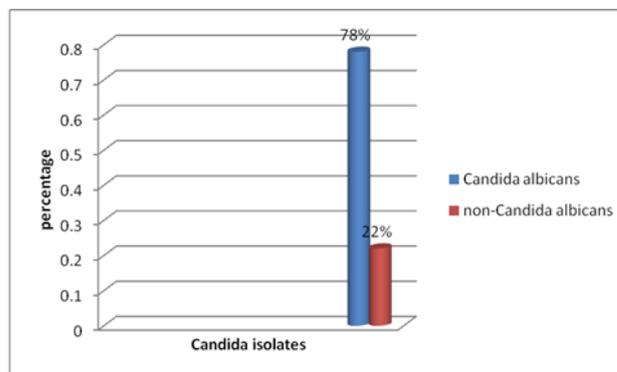


Figure 1. Percentage of *Candida albicans* against non *Candida albicans* isolates detected on positive yeast cultures

Table 1. Vitek2 compact system identification results

Isolate number	Identified species	probability	confidence
1	<i>Candida lusitaniae</i>	98 %	Excellent
2	<i>Candida lusitaniae</i>	96%	Excellent
3	<i>Candida lusitaniae</i>	97 %	Excellent
4	<i>Candida lusitaniae</i>	97 %	Excellent
5	<i>Candida lusitaniae</i>	98 %	Excellent
6	<i>Candida lusitaniae</i>	96 %	Excellent
7	<i>Candida lusitaniae</i>	98 %	Excellent
8	<i>Candida lusitaniae</i>	97%	Excellent
9	<i>Candida lusitaniae</i>	97 %	Excellent

Table 2. Antifungal susceptibility of *Candida lusitaniae* isolates using AST cards of Vitek2 Compact system

Drug	MIC (µg/ml)	Interpretation
Fluconazole	≤ 1	S
Flucytosine	≤ 1	S
Voriconazole	≤ 0.12	S
Amphotericin B	≥ 2	R

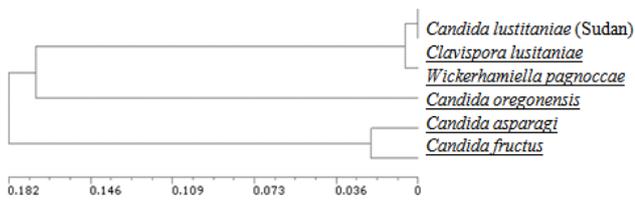


Figure 2. Phylogenetic tree of *Candida lusitaniae* Sudan isolates and other *Candida* species that obtained from database

4. Discussion

This study documented that there are new emergent species of *Candida* should be considered when dealing with specimen collected from patients suspected for yeast infections. Identification of *C. lusitaniae* has proven to be problematic, with many earlier reported cases being misidentified initially as *Candida parapsilosis* or *Candida tropicalis* or even *Saccharomyces* species. [9,10,11,12]. The morphologic characteristics, fermentation and assimilation reactions, and growth on selected media have been well documented for this species [13]. The antifungal susceptibility test agrees with the previous studies [11,14,15] that reported the resistance of *C. lusitaniae* to Amphotericin B. Our results provide useful information that *C. lusitaniae* can be isolated and identified as well as other *Candida* species from immunocompromised patients in Sudan.

5. Conclusion

We concluded that *C. lusitaniae* should be considered an opportunistic pathogen in both adults and children when isolated in the appropriate clinical setting, especially from patients with underlying malignancy undergoing cytotoxic therapy, with granulocytopenia receiving prolonged broad-spectrum antibiotic therapy, or with intravascular catheters. All isolates of *C. lusitaniae* should undergo susceptibility testing both before and during therapy, since the occurrence of amphotericin B resistance has been frequently reported. In addition, clinical isolates suspected of being new strain of *Candida* should undergo biochemical testing, such as the API 20C system, additional assimilation and fermentation tests and molecular identification to ensure that misidentification does not occur.

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Competing Interests

There were no conflicts of interest.

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